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SPECIFICATION FOR CASEIN (EDIBLE QUALITY)

(Revised)

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

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SPECIFICATION FOR CASEIN (EDIBLE QUALITY)

(Revised)

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AMENDMENT NO. 1 DECEMBER 1989
TO
IS : 1167 - 1965 SPECIFICATION FOR CASEIN (EDIBLE QUALITY)

[*Page* 4, *Table* 1, *Sl No.* (ii), *col* 3] — Substitute ' 3·0 ' *for* ' 2·5 '.

[*Page* 4, *Table* 1, *Sl No.* (vii), *col* 3] — Substitute ' 6·0 ' *for* ' 5·6 '

(AFDC 34)

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Indian Standard

SPECIFICATION FOR CASEIN (EDIBLE QUALITY)

(Revised)

0. FOREWORD

0.1 This Indian Standard (Revised) was adopted by the Indian Standards Institution on 30 September 1965, after the draft finalized by the Dairy Industry Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 Casein is the chief protein of milk and it exists as a colloidal suspension. It is manufactured from wholesome skim milk by selective precipitation, and removal of whey, subsequent washing and drying of the precipitate.

0.3 Since casein is a good protein nutritionally, it is used in the manufacture of protein foods for oral administration in cases of protein malnutrition, gastric disorders and other symptoms which call for protein supplementation.

0.4 The Indian Standards Institution has already published a specification for natural sour (lactic) casein for glue manufacture (IS: 850-1957) which covers the material produced by natural sour (lactic acid) precipitation, meant specially for glue manufacture. But there has been a long felt need for a standard to cover casein which is used for manufacture of edible products.

0.5 The Indian Standard specification for casein (edible quality) (IS: 1167-1957) was published in 1957. The need for revising the standard was felt because the original version covered material prepared by acid precipitation method only. The scope has now been enlarged to embrace products prepared by acid precipitation or natural souring. The limit for moisture content has been increased so that the standard would be more realistic. Further, additional requirements, such as total acidity, free acidity and total bacterial count, have been introduced in this revision.

0.6 For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS: 2-1960*. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

---

*Rules for rounding off numerical values (*revised*).

1. SCOPE

1.1 This standard prescribes the requirements and the methods of test for casein of edible quality.

2. REQUIREMENTS

2.1 Casein shall be prepared from skim milk of either cow or buffalo or a mixture of both. Casein shall be nearly white or pale cream in colour and shall have no undesirable odour or any foreign matter (*see* Appendix L). It shall be free from any added colour or preservative.

2.2 Particle Size — The size of the particles shall be such that 100 percent by weight of casein shall pass through 500-micron IS Sieve (*see* IS : 460-1962*).

NOTE — The aperture of BS Sieve 30 is within the limits laid down for the specified IS test sieve and may, therefore, be used as 500-micron IS Sieve.

2.3 Hygienic Requirements — Casein shall be manufactured and stored in premises built and maintained under hygienic conditions (*see* IS : 2491-1963†).

2.4 The material shall also comply with the requirements given in Table 1.

TABLE 1 REQUIREMENTS FOR CASEIN (EDIBLE QUALITY)

SL No.	CHARACTERISTIC	REQUIREMENT	METHOD OF TEST (REF TO APPENDIX)
(1)	(2)	(3)	(4)
i)	Moisture, percent by weight, *Max*	10·0	A
ii)	Total ash, percent by weight (on dry basis), *Max*	2·5	B
iii)	Acid insoluble ash, percent by weight (on dry basis) *Max*	0·1	C
iv)	Fat, percent by weight (on dry basis), *Max*	1·5	D
v)	Nitrogen, percent by weight (on dry basis), *Min*	14·5	E
vi)	Total acidity in terms of ml of 0·1 N NaOH/g	6 to 14	F
vii)	Free acidity in terms of ml of 0·1 N NaOH/10 g, *Max*	5·6	G
viii)	Bacterial count, per gram, *Max*	50 000	H
ix)	Coliform count, per gram, *Max*	10	J
x)	Mould count, per gram, *Max*	50	K
xi)	Rate of solubility	To conform to test	L

---

*Specification for test sieves (*revised*).

†Code for sanitary conditions for food processing units. (Since revised).

3. PACKING AND MARKING

3.1 Packing — The material shall be packed in such a way as to protect it from contamination and deterioration. Polyethylene lined bags are recommended.

3.1.1 Unless otherwise agreed to between the purchaser and the vendor, the packages shall be of 20 or 50 kg nett of material.

3.2 Marking — The following information shall be marked legibly and indelibly on each container:

a) Name of the material;
b) Name and address of the manufacturer;
c) Batch or code number; and
d) Net weight.

3.2.1 The product may also be marked with Standard mark.

3.2.2 The use of the Standard Mark is governed by the provisions of the *Bureau of Indian Standards Act*, 1986 and the Rules and Regulations made thereunder. The details of conditions under which the licence for the use of Standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

4. SAMPLING

4.1 Representative samples of the material shall be drawn and tested for conformity to this standard as described in Appendix M.

5. TESTS

5.1 Tests shall be carried out as prescribed in the appropriate appendices given in col 4 of Table 1.

5.2 Quality of Reagents — Unless specified otherwise, pure chemicals shall be employed in tests, and distilled water shall be used where the use of water as a reagent is intended.

NOTE — ' Pure chemicals ' shall mean chemicals that do not contain impurities which affect the results of analysis.

APPENDIX A

[*Table* 1, *Item* (i)]

DETERMINATION OF MOISTURE

A-1. PROCEDURE

A-1.1 Weigh accurately about 3 g of the material, powdered if necessary, into a flat bottomed glass or aluminium dish (with a cover) previously dried and weighed. Heat the dish containing the material (after uncovering) in an electric oven maintained at 100° to 102°C for about 3 hours. Cool in a desiccator and weigh with the cover on. Repeat the process of drying, cooling and weighing at half hourly intervals, until the difference between two consecutive weighings is less than one milligram. Preserve the dish containing this dried material in a desiccator for the determination of total ash (**B-1.1**).

A-2. CALCULATION

A-2.1 Moisture, percent by weight $= \frac{100(W_1 - W_2)}{W_1 - W}$

where

W_1 = weight in g of the dish with material before drying,
W_2 = weight in g of the dish with material after drying, and
W = weight in g of the empty dish.

APPENDIX B

[*Table* 1, *Item* (ii)]

DETERMINATION OF TOTAL ASH

B-1. PROCEDURE

B-1.1 Heat gently the crucible containing the dried material (**A-1.1**) on the flame and then in a muffle furnace at 550° ± 20°C till grey ash results. Cool the crucible in a desiccator and weigh. Repeat the process of heating in the muffle furnace for 30 minutes, cooling and weighing until the difference between two consecutive weighings is less than 1 mg. Note the lowest weight. Preserve the dish with the contents for the determination of acid insoluble ash (**C-2.1**).

B-2. CALCULATION

B-2.1 Total ash, percent by weight (on dry basis) $= \frac{100\,(W_1 - W)}{W_2 - W}$

where

W_1 = weight in g of the crucible containing the ash,
W = weight in g of the crucible, and
W_2 = weight in g of the crucible with the dried material taken for the test.

APPENDIX C

[*Table* 1, *Item* (iii)]

DETERMINATION OF ACID INSOLUBLE ASH

C-1. REAGENT

C-1.1 Hydrochloric Acid — approximately 5 N

C-2. PROCEDURE

C-2.1 To the ash contained in the porcelain dish (**B-1.1**) add 25 ml hydrochloric acid, cover with a watch-glass and heat on a water-bath for 10 minutes. Allow to cool and filter the contents of the dish through a Whatman filter paper No. 42 or its equivalent. Wash the filter with water until the washings are free from the acid. Return the filter and the residue to the dish. Keep it in an electric air-oven maintained at 135° ± 2°C for about 3 hours. Ignite in a muffle furnace at 550° ± 20°C for one hour. Cool the dish in a desiccator and weigh. Repeat the process of igniting in the muffle furnace, cooling and weighing at half-hour intervals until the difference between two successive weighings is less than one milligram. Note the lowest weight.

C-3. CALCULATION

C-3.1 Acid insoluble ash, percent by weight (on dry basis) $= \frac{100\,(W_2 - W)}{W_1 - W}$

where

W_2 = weight in g of the porcelain dish with the acid insoluble ash,
W = weight in g of the empty porcelain dish, and
W_1 = weight in g of the porcelain dish with the dried material taken for the determination of total ash (**B-1.1**).

APPENDIX D

[*Table* 1, *Item* (iv)]

DETERMINATION OF FAT

D-1. APPARATUS

D-1.1 Fat Extraction Apparatus — conforming to IS: 2311-1963*.

D-2. REAGENTS

D-2.1 Hydrochloric Acid — density 1·122 g/ml at 20°C.

D-2.2 Ethyl Ether

D-2.3 Petroleum Ether — redistilled slowly at a temperature below 65°C.

D-3. PROCEDURE

D-3.1 Place 10 ml of hydrochloric acid in a 100-ml beaker. Carefully transfer to the beaker about 5 g of the material accurately weighed. Add about 10 ml of the hydrochloric acid so as to wet and wash down any particles of the material that might have adhered to the sides. Cautiously heat the contents over a burner making sure that all particles of the material adhering to the sides are dissolved in the acid. Finally boil for 10 minutes. Cool the beaker until a temperature of approximately 20°C is reached. Quantitatively transfer the contents to a Mojonnier flask or a Rohrig tube using about 10 ml of acid as wash liquid. Add 25 ml of ethyl ether to the beaker and transfer the contents of the Mojonnier flask or the Rohrig tube. Stopper with cork or a stopper of synthetic rubber unaffected by usual fat solvents, and shake vigorously for one minute. First add 25 ml of petroleum ether to the beaker and then transfer it to the extraction flask or tube. Repeat vigorous shaking. Centrifuge the Mojonnier flask at about 600 rotations per minute. If a Rohrig tube is used, let it stand until the upper liquid is practically clear. Decant the ether solution into a suitable flask or metal dish. Wash the lip and the stopper of extraction flask or tube with a mixture of equal parts of both the solvents and add the washings to the weighing flask or metal dish. Repeat extraction of the liquid remaining in flask or tube twice, using 15 ml of each solvent each time. Evaporate the solvent completely on a hot-plate or steam-bath at a temperature that does not cause spattering or bumping. Dry the fat in an oven at the temperature of boiling water to constant weight. Weigh the cooled flask or metal dish, using counterpoise duplicate container handled in the same manner. Remove the fat completely from the container with warm petroleum ether and weigh as before.

---

*Specification for fat extraction apparatus for milk and milk products (**Since revised**).

D-4. CALCULATION

D-4.1 Fat, percent by weight (on dry basis) = $\frac{10\,000\,(W_1 - W_2)}{W_3\,(100 - m)}$

where

W_1 = weight in g of contents in flask or metal dish before removal of fat,

W_2 = weight in g of contents in flask or metal dish after removal of fat,

W_3 = weight in g of the material taken for the test, and

m = percentage by weight of moisture in the material (**A 2.1**).

APPENDIX E

[*Table* 1, *Item* (v)]

DETERMINATION OF NITROGEN

E-1. APPARATUS

E-1.1 A recommended apparatus, as assembled, is shown in Fig. 1.

E-1.1.1 *Description* — The apparatus consists of a round bottomed flask *A* of 1 000 ml capacity fitted with a rubber stopper through which passes one end of the connecting bulbs tube *B*. The other end of the bulb tube *B* is connected to the condenser *C* which is attached by means of a rubber tube to a dip tube *D* which dips into the liquid contained in a beaker *E* of 250 ml capacity.

E-2. REAGENTS

E-2.1 Anhydrous Sodium Sulphate

E-2.2 Copper Sulphate

E-2.3 Concentrated Sulphuric Acid — sp gr 1·84.

E-2.4 Sodium Hydroxide Solution — Dissolve about 225 g of sodium hydroxide in 500 ml of water.

E-2.5 Standard Sulphuric Acid — approximately 0·1 N.

E-2.6 Methyl Red Indicator Solution — Dissolve one gram of methyl red in 200 ml of rectified spirit (95 percent by volume).

E-2.7 Standard Sodium Hydroxide Solution — approximately 0·1 N.

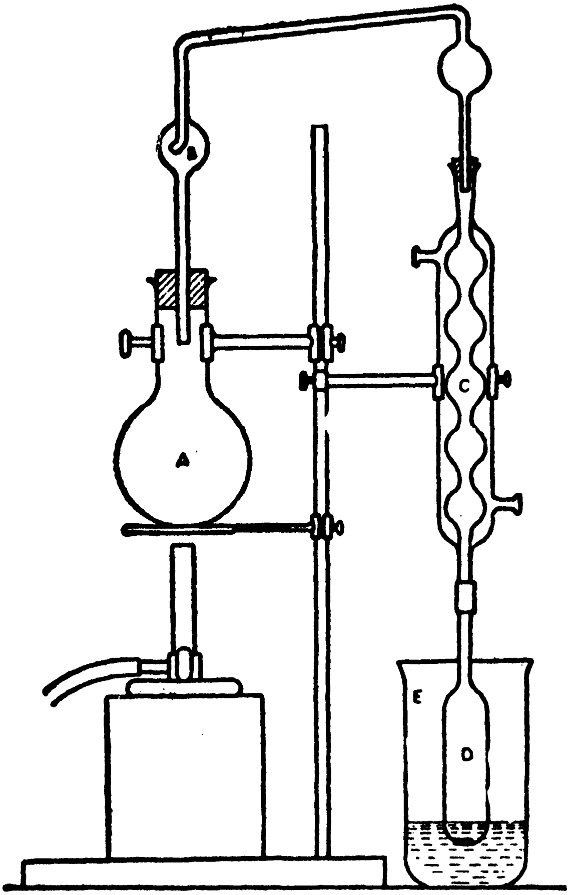

FIG. 1 APPARATUS FOR DETERMINATION OF NITROGEN

E-3. PROCEDURE

E-3.1 Transfer carefully 0·2 g of the material, accurately weighed, to a Kjeldahl flask, taking precaution to see that particles of the material do not stick in the neck of the flask. Add about 15 to 18 g of anhydrous sodium sulphate, about 0·2 g of copper sulphate and 25 ml of concentrated sulphuric acid. Place the flask in an inclined position. Heat below the boiling point of the acid until frothing ceases. Increase heat until the acid boils vigorously and digest for 30 minutes after the mixture becomes clear and pale green or colourless. Wash down with the minimum quantity of concentrated sulphuric acid, particles, if any, sticking to the sides and continue digestion for 60 to 90 minutes. Cool the contents of the flask. Transfer quantitatively, using water, to the round bottom flask *A* and dilute to 250 ml. Add with shaking, a few pieces of pumice stone to prevent bumping. Add about 60 ml of sodium hydroxide solution or more, if necessary (to make the solution alkaline) carefully through the side of the flask so that it does not mix at once with the acid solution but forms a layer below it. Assemble the apparatus as shown in Fig. 1, taking care that the dip tube *D* extends below the surface of a known quantity of standard sulphuric acid contained in the beaker *E*. Mix the contents of the flask by shaking and distil until all ammonia has passed over into the standard sulphuric acid. Detach flask *A* from the condenser and shut off the burner. Rinse the condenser thoroughly with water into the beaker *E*. Wash the dip tube *D* carefully so that all traces of the condensate are transferred to the beaker. When all the washings have drained into the beaker *E*, add two or three drops of methyl red indicator and titrate with standard sodium hydroxide solution.

E-3.2 Carry out a blank using all reagents in the same quantities but without the material to be tested.

E-4. CALCULATION

E-4.1 Nitrogen, percent by weight $= \frac{140\,(B - A)\,N}{W\,(100 - m)}$

where

B = volume in ml of standard sodium hydroxide solution used to neutralize the acid in the blank determination,

A = volume in ml of standard sodium hydroxide solution used to neutralize the excess of acid in the test with the material,

N = normality of standard sodium hydroxide,

W = weight in g of the material taken for the test, and

m = percentage by weight of moisture in the material (**A-2.1**).

APPENDIX F

[*Table* 1, *Item* (vi)]

DETERMINATION OF TOTAL ACIDITY

F-1. REAGENTS

F-1.1 Standard Sodium Hydroxide Solution — 0·1 N.

F-1.2 Standard Sulphuric Acid — 0·1 N.

F-1.3 Bromothymol Blue Indicator Solution — 0·04 percent (*w/v*) in rectified spirit.

F-2. PROCEDURE

F-2.1 Transfer 2 g of the sample to a 250-ml flask. Add 100 ml of freshly boiled and cooled distilled water and soak for 15 minutes. Add 50 ml of 0·1 N sodium hydroxide solution and close the mouth of the flask. Heat in a water-bath at 50°C and shake until the sample has been completely dissolved. Titrate with standard sulphuric acid, using 1 ml of 0·04 percent solution of bromothymol blue as indicator. Carry out the determination omitting the casein and make a suitable correction. The acidity is expressed as the number of millilitres of 0·1 N sodium hydroxide solution required to neutralize 1·0 g of sample.

APPENDIX G

[*Table* 1, *Item* (vii)]

DETERMINATION OF FREE ACIDITY

G-1. REAGENTS

G-1.1 Standard Sodium Hydroxide Solution — 0·1 N.

G-1.2 Phenolphthalein Indicator Solution — prepared by dissolving 0·1 g of phenolphthalein in 100 ml of 60 percent rectified spirit (*see* IS: 2263-1962*).

G-2. PROCEDURE

G-2.1 Weigh 20 g of casein sample to within 0·1 g and transfer to a 500-ml beaker containing 200 ml of boiling distilled water. Cover the beaker with a watch-glass while allowing the mixture to stand for 30 minutes with frequent agitation. Filter the aqueous layer and pipette 50 ml of the cold filtrate (corresponding to 5 g of casein) into a flask. Titrate with standard sodium hydroxide solution using phenolphthalein indicator. The free acidity is expressed as the number of millilitres of 0·1 N sodium hydroxide solution required to neutralize 10 g of sample.

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*Methods of preparation of indicator solutions for volumetric analysis.

APPENDIX H

[*Table* 1, *Item* (viii)]

DETERMINATION OF BACTERIAL COUNT

H-1. APPARATUS

H-1.1 Weighing Scoop, Sterile — with counter weight.

H-1.2 Bacteriological Transfer Pipettes, Sterile — accurately graduated, with cotton plug in the upper orifice.

H-1.3 Dilution Bottles, Sterile — made of heat resistant glass (preferably borosilicate glass) of the following capacities:

a) 150 ml, with mark at 99 ml level; and
b) 25 ml, with mark at 9 ml level.

H-1.4 Petri Dishes — with outside dish diameter 100 mm, inside dish diameter 91 mm, and depth 15 mm. The exterior and interior surfaces of the bottoms should be flat and free from bubbles, scratches or other defects.

H-1.5 Bacteriological Tubes, Sterile — 25 ml capacity with a mark at the 10 ml level, with cotton plugs.

H-2. REAGENTS

H-2.1 Dilution Waters — There shall be two types of dilution water, namely, Dilution Water 1 for use with Medium 1 (**H-2.2.1**) and Dilution Water 2 for use with Medium 2 (**H-2.2.2**). Sterilize dilution water at 121°C for 15 minutes before use.

H-2.1.1 *Dilution Water* 1 — Dissolve 34 g of potassium dihydrogen phosphate (KH_2PO_4) in 500 ml of distilled water, adjust to *p*H 7·2 with 1 N sodium hydroxide solution and make up to one litre with distilled water. Dilute 1·25 ml of this stock phosphate buffer solution with water to one litre to obtain dilution water.

H-2.1.2 *Dilution Water* 2 — shall have the following composition:

Sodium chloride	9 g
Potassium chloride	0·42 g
Calcium chloride	0·24 g
Sodium bicarbonate	0·2 g
Distilled water	1 000 ml

H-2.2 Media — Either of the two media given in **H-2.2.1** and **H-2.2.2** shall be used.

H-2.2.1 *Medium* 1 — shall have the following composition:

Peptone	5·0 g
Yeast extract	2·5 g
Glucose (dextrose)	1·0 g
Agar, bacteriological grade	20·0 g
Distilled water	1 000 ml

H-2.2.1.1 *Preparation* — Soak the materials (**H-2.2.1**) for 3 to 5 minutes in cold water, then bring the mixture into complete solution with minimum delay by boiling above an asbestos centred wire gauze over a flame. Stir continuously and efficiently to avoid charring. Adjust the solution to *p*H 7·0 at 50°C with sodium hydroxide solution. Filter through cotton pad till clear filtrate is obtained. Fill into bacteriological tubes to 10-ml mark. Sterilize in an autoclave at 121°C for 20 minutes.

H-2.2.2 *Medium* 2 — shall have the following composition:

Yeastrel	3 g
Peptone	5 g
Agar, bacteriological grade	20 g
Fresh whole milk	10 ml
Distilled water	1000 ml

H-2.2.2.1 *Preparation* — Dissolve the yeastrel and the peptone in distilled water in the steamer and adjust the reaction at room temperature to *p*H 7·4 using phenol red as the indicator. Wrap shreded agar in muslin and wash in running cold water for 15 minutes. Squeeze out the excess cold water and add the agar together with the freshly shaken milk to the broth. Autoclave at 121°C for 20 minutes and filter through paper pulp (*see* Note) in a Buchner funnel. (Egg should not be used for clearing.) Take the agar directly from the autoclave and filter hot, the whole apparatus being kept warm by a surrounding atmosphere of steam. Test the reaction of the filtrate at 50°C and adjust, if necessary, to *p*H 7·0, so that the final reaction at room temperature is *p*H 7·2.

Fill this medium into bacteriological tubes to 10-ml mark. Sterilize in an autoclave at 121°C for 20 minutes.

NOTE — The paper pulp is prepared by pulping small pieces of postlip or white heather paper in water by means of a pestle and mortar. A single layer of Chardin filter paper should be laid on top of the Buchner funnel to prevent the pulp being sucked through, and the pulp itself should then be packed down evenly on top of it. The filter when ready for use, should have a total depth of about 1·5 mm.

H-3. PROCEDURE

H-3.1 Dilution — Weigh 11 g of the material from the sample marked ' For Bacteriological Tests ' (**M-3.1**) using a sterile spatula and suspend in 99 ml of dilution water at 45°C. Agitate mildly, soak for 1 to 3 minutes and then agitate vigorously to avoid churning out the fat. Prepare dilutions of this so as to give between 30 and 300 colonies per plate (*see* **H-3.5**). Add one millilitre of suitable dilutions in triplicate to the sterile petri dishes. Use at least 5 dilutions.

H-3.2 Pouring Plates — Melt agar medium (**H-2.2**) in bacteriological tubes and keep at 48° to 50°C. Introduce this medium aseptically, at 42° to 44°C, into the petri dishes and mix by rotating and tilting dishes without spreading over the edges. Spread the mixture evenly over the bottom of the plate. Allow to solidify.

H-3.3 Incubation — Invert the plates and incubate at 37°C for 48 hours.

H-3.4 Counting — Count the colonies with the aid of magnification under uniform and properly controlled illumination. Count only those plates with 30 to 300 colonies, including pin point size colonies.

H-3.5 Computation — Compute the bacterial count per gram from the dilutions used.

NOTE — All precautions shall be observed to prevent microbiological contamination throughout the test.

APPENDIX J

[*Table* 1, *Item* (ix)]

DETERMINATION OF COLIFORM COUNT

J-1. GENERAL

J-1.1 Coliform Bacteria — Coliform bacteria includes all aerobic and facultative anaerobic, Gram-negative, nonspore — forming bacteria which ferment lactose with the production of acid and gas. A positive presumptive test is indicated by the formation of acid and gas within 48 hours at 35° to 37°C in a fermentation tube containing lactose bile salt broth. Alternatively, the development of dark red colonies at least 0·5 mm in diameter in a solid medium (violet and red bile agar) within 20 to 24 hours at 35° to 37°C may be considered as a positive evidence of the presence of coliform bacteria. Violet red bile agar is one of the standard media used for determination of general types of coliform organisms including those of faecal origin in water, milk and other materials of sanitary importance.

J-2. APPARATUS

J-2.1 Weighing Scoop, Sterile — with counter weight.

J-2.2 Bacteriological Transfer Pipettes, Sterile — accurately graduated, with cotton plug in the upper orifice.

J-2.3 Dilution Bottles, Sterile — made of heat-resistant glass (preferably borosilicate glass) closed with rubber stoppers, preferably screw caps with new friction-fit liners for making them leak-proof and of the following capacities:

a) 150 ml, with mark at 99 ml level; and

b) 25 ml, with mark at 9 ml level.

J-2.4 Petri Dishes — with outside dish diameter 100 mm, inside dish diameter 91 mm, and depth 15 mm. The exterior and interior surfaces of the bottom should be flat and free from bubbles, scratches or other defects, which would interfere with counting of colonies.

J-2.5 Bacteriological Tubes, Sterile — 25 ml capacity with a mark at the 10-ml level, with cotton plugs.

J-2.6 Tubes having Inverted Vials — Durham tubes.

J-3. REAGENTS

J-3.1 Dilution Water — Dissolve 34 g of potassium dihydrogen phosphate (KH_2PO_4) in 500 ml of distilled water, adjust to *p*H 7·4 with 1 N sodium hydroxide solution and make up to one litre with distilled water. Dilute 1·25 ml of this stock phosphate buffer solution with distilled water to one litre to obtain dilution water.

J-3.2 Medium — Violet red bile agar shall be the medium, the composition of which shall be as follows:

Yest extract	3·0 g
Peptone	7·0 g
Sodium taurocholate	1·5 g
Lactose	10·0 g
Sodium chloride	5·0 g
Agar-agar	20·0 g
Indicator:	
Neutral red	0·03 g
Crystal violet	0·002 g
Water	1 000 ml
Final *p*H	7·4±0·1

J-3.2.1 *Preparation and Sterilization of Medium* — Soak the materials (**J-3.2**) for 3 to 5 minutes in cold water, then bring the mixture into complete solution with minimum delay by boiling above an asbestos-centred wire gauze, over a flame. Stir continuously and efficiently to avoid charring. Adjust the solution to *p*H 7·4±0·1 at 50°C with sodium hydroxide solution. Filter through cotton pad till clear filtrate is obtained. Fill the filtrate into bacteriological tubes to 10-ml mark. Sterilize in an autoclave at 121°C for 15 minutes.

J-4. PROCEDURE

J-4.1 Dilution — Using aseptic precautions and appropriate means thoroughly mix each sample until representative portions may be removed. Heat 99 ml of sterile 2 percent sodium citrate solution into a sterile container (preferably 150 ml wide-mouth screw-cap jars) to 45°C for dispersing and diluting casein. Promptly add 11 g of casein and triturate it with sterile glass rod until thoroughly mixed. Allow the mixture to cool at room temperature. Prepare suitable dilutions of this and add one millilitre of suitable dilutions in triplicate to the sterile petri dishes.

J-4.2 Pouring Plates — Melt the medium (**J-3.2.1**) in bacteriological tubes and keep at 48° to 50°C. Introduce this medium aseptically at 42° to 44°C into the petri dishes and mix by rotating and tilting dishes without spreading over the edges. Spread the mixture evenly over the bottom of the plate. Allow to solidify. After solidification of medium in plate, add cover layer of medium.

J-4.3 Incubation — Invert the plates and incubate at 35° to 37°C for 24 hours.

J-4.4 Counting — Count the dark red colonies which have a diameter of 0·5 mm or over.

J-4.5 Computation — Compute the coliform count per gram from the dilutions used (**J-4.1**).

NOTE 1 — In case of doubt regarding the colonies developed on violet red bile agar, representative colonies are picked and transferred to lactose bile salt broth in tubes having inverted vials. Production of acid and gas is confirmatory for coliform organisms.

NOTE 2 — All precautions shall be observed to prevent microbiological contamination throughout the test.

APPENDIX K

[*Table* 1, *Item* (x)]

DETERMINATION OF MOULD COUNT

K-1. GENERAL

K-1.1 Mould — High mould count is not desirable and indicates that casein is manufactured under improper plant sanitary control. It is also a result of improper packing and faulty storage. The development of colonies in a solid medium (potato-dextrose-agar at *p*H 3·5) within 5 days at 22°C should be considered as a positive evidence of moulds.

K-2. APPARATUS

K-2.1 Weighing Scoop, Sterile — with counter weight.

K-2.2 Bacteriological Transfer Pipettes, Sterile — accurately graduated, with cotton plug in the upper orifice.

K-2.3 Dilution Bottles, Sterile — made of heat-resistant glass (preferably borosilicate glass) closed with rubber stoppers, preferably screw caps with new friction-fit liners for making them leak-proof and of the following capacities:

a) 150 ml, with mark at 99 ml level; and
b) 25 ml, with mark at 9 ml level.

K-2.4 Petri Dishes — with outside dish diameter 100 mm, inside dish diameter 91 mm, and depth 15 mm. The exterior and interior surfaces of the bottom should be flat and free from bubbles, scratches or other defects, which would interfere with counting of colonies.

K-2.5 Bacteriological Tubes, Sterile — 25 ml capacity with a mark at 10 ml level, with cotton plugs.

K-3. REAGENTS

K-3.1 Dilution Water — Dissolve 34 g of potassium dihydrogen phosphate (KH_2PO_4) in 500 ml of distilled water, adjust to *p*H 7·4 with 1 N sodium hydroxide solution and make up to one litre with distilled water. Dilute 1·25 ml of this stock phosphate buffer solution with distilled water to one litre to obtain dilution water.

K-3.2 Medium — Potato glucose agar (acidified) shall be the medium, the composition of which shall be as follows:

Potato infusion	From 200 g of white potatoes (*see* Note)
Agar	15 g
Glucose	20 g
Distilled Water	1 000 ml

NOTE — Suspend 200 g of sliced potatoes in distilled water sufficient to cover the material. Boil for one hour and filter through cheese cloth.

K-3.2.1 *Preparation and Sterilization of Medium* — Heat the mixture (**K-3.2**) to boiling to dissolve the ingredients. Distribute into tubes or flasks and autoclave for 15 minutes at 121°C. Melt in flowing steam or boiling water, and cool. Adjust reaction in each container to *p*H 3·5±0·1 with sterile 10 percent tartaric or lactic acid. As remelting of acidified medium may destroy its solidifying properties, adjust only amount needed for immediate plating. Amount of acid required for adjustment in any one flask of same batch of medium ordinarily will establish amount needed in each of the other flask containing equal quantities.

For colorimetric adjustment, use bromophenol blue and titrate 5 ml of medium with dilute acid prepared by adding 1 ml of sterile 10 percent stock solution to 19 ml of water. The number of millilitres of dilute acid used to titrate to *p*H 3·5 will represent the amount of stock solution that shall be added to 100 ml of medium.

K-4. PROCEDURE

K-4.1 Dilution — Using aseptic precautions and appropriate means, thoroughly mix each sample until representative portions may be removed. Heat 99 ml of sterile 2 percent sodium citrate solution into a sterile container (preferably 150 ml wide-mouth screw-cap jars) to 45°C for dispersing and diluting casein. Promptly add 11 g of casein and triturate it with sterile glass rod until thoroughly mixed. Allow the mixture to cool at room temperature. Prepare suitable dilutions of this and add one millilitre of suitable dilutions in triplicate to the sterile petri dishes.

K-4.2 Pouring Plates — Melt the medium (**K-3.2.1**) in bacteriological tubes and keep at 48° to 50°C. Introduce this medium aseptically at 42° to 44°C into the petri dishes and mix by rotating and tilting dishes without spreading over the edges. Spread the mixture evenly over the bottom of the plate. Allow to solidify. After solidification of the medium in plate, add cover layer of medium.

K-4.3 Incubation — Invert the plates and incubate at 22°C for 5 days.

K-4.4 Counting — If moulds are developing in large numbers, count plates on the third day of incubation and then recount on the fifth day.

K-4.5 Computation — Compute the mould count per gram from the dilutions used (*see* **K-4.1**).

APPENDIX L

[*Table* 1, *Item* (xi)]

DETERMINATION OF RATE OF SOLUBILITY

L-1. APPARATUS

L-1.1 Glass Stirring Rods — with flattened ends.

L-1.2 Centrifuge Tubes — conical, approximately 170 mm long and 25 mm diameter, graduated at 50 ml and at 0·1 ml intervals between 0 and 1 ml and at 0·2 ml intervals between 1 and 3 ml.

L-1.3 Centrifuge — with swing-out buckets and capable of a speed to give approximately 900 revolutions per minute.

L-2. REAGENTS

L-2.1 Borax Solution — dissolve 20 g of sodium tetraborate ($Na_2B_4O_7$, $10H_2O$) in water and dilute to 1 000 ml.

L-2.2 Water — *p*H 6·0 at 20°C.

L-2.3 Sodium Hydroxide Solution — 1·0 N.

L-3. PROCEDURE

L-3.1 Weigh 6 g of the casein sample in a test-tube containing a glass stirring rod. Add 36 ml of borax solution in the test-tube, stir and mix the contents with the rod. Add a volume of 0·1 N sodium hydroxide solution equivalent to the 'Free acidity', (*see* **G-2.1**). Heat the tube in a water-bath at 70°±1°C with continuous mixing of the contents for the first 5 minutes, and then at an interval of every 5 minutes for a period of 40 minutes. If the casein dissolves completely, note the time of complete solution. If after 45 minutes the casein is not completely dissolved, transfer the contents of the test-tube to a centrifuge tube, wash the test tube with water at 70°C and collect the washings until the volume in the centrifuge tube becomes 50 ml. Centrifuge at a speed of about 900 rev/min for 10 minutes. Decant the supernatant liquid, including any light material which may be present above the compact, lower layer of sediment. Add 40 ml of cold water, mix and centrifuge again for 10 minutes at 900 rev/min. Read the volume of sediment excluding any light material above the compact layer.

L-4. REPORT

L-4.1 For conformity, the casein sample shall not have sediment which is indication of the presence of insoluble material.

APPENDIX M

(*Clause* 4.1)

SAMPLING OF EDIBLE CASEIN

M-1. GENERAL REQUIREMENTS OF SAMPLING

M-1.0 In drawing, preparing storing and handling samples the following precautions and protections shall be observed.

M-1.1 Samples shall be taken in a protected place not exposed to damp air, dust or soot.

M-1.2 The sampling instrument shall be clean and dry when used. When taking samples for bacteriological examination, it shall also be sterile.

M-1.3 The samples, the material being sampled, the sampling instrument and the containers for sampling shall be protected from adventitious contamination.

M-1.4 The samples shall be placed in clean and dry glass containers. The sample containers shall be of such a size that they are almost completely filled by the sample. The sample containers shall, in addition, be sterile when they are used for samples for bacteriological examination.

M-1.5 Each sample container shall be sealed air-tight after filling and marked with full details of sampling, batch or code number, name of the manufacturer and other important particulars of the lot.

M-1.6 The samples shall be stored in such a manner that the temperature of the material does not vary unduly from the normal temperature and that they are protected from light.

M-1.7 Sampling shall be done by a person agreed to between the purchaser and the vendor and in the presence of the purchaser (or his representative) and the vendor (or his representative).

M-2. SCALE OF SAMPLING

M-2.1 Lot — In a single consignment all the containers containing the material of the same grade and the same batch of manufacture shall constitute a lot.

M-2.2 Sample shall be tested for each lot for ascertaining the conformity of the material for the requirements of this specification.

M-2.3 The number of containers to be selected from a lot shall depend on the size of the lot and shall be in accordance with col 1 and 2 of Table 2.

M-2.4 These containers shall be chosen at random and for this purpose a random number table as agreed to between the purchaser and the vendor shall be used. In case such a table is not available the following procedure shall be adopted:

> Starting from any container, count all the containers in one order as 1, 2, 3, ... up to r and so on. Every rth container so counted shall be withdrawn, where r is the integral part of N/n, N being the number of containers in the lot and n the number of containers to be selected.

M-3. PREPARATION OF SAMPLES

M-3.1 Individual Samples for Bacteriological Examination — Draw with an appropriate sampling instrument, which is sterile, about 300 g

TABLE 2 NUMBER OF CONTAINERS TO BE SELECTED FOR SAMPLING

(*Clause* M-2.3)

NUMBER OF CONTAINERS IN THE LOT N	NUMBER OF CONTAINERS TO BE SELECTED n
1	1
2 to 8	2
9 „ 25	3
26 „ 50	4
51 „ 300	5
301 „ 500	6
501 and over	8

of the material from each selected container by taking small portions of the material from at least 10 different parts of the container. Thoroughly mix all portions of the material drawn from the same container. Take about 100 g of the material and divide this material into three equal parts taking care not to bring any bacterial contamination. Each part so obtained shall constitute an individual sample representing the consignment and shall be transferred to sterile glass containers which shall be sealed air-tight and labelled with the particulars described in **M-1.5** and the words ' For Bacteriological Tests '. The individual samples so obtained shall be divided into three sets in such a way that each set has a sample representing each selected container. One of these sets shall be marked for the purchaser, another for the vendor and the third for the referee.

M-3.2 Composite Samples for Other Tests — From the mixed material from each selected container remaining after the individual sample has been taken, equal quantities of the material from each container, shall be taken and mixed together so as to give material representative of the lot and weighing not less than 200 g. This material shall be divided into three equal parts. Each part so obtained shall constitute a composite sample representing the lot and shall be transferred to a clean, dry container made of glass or tinplate and labelled with the particulars given in **M-1.5**. One of these composite samples shall be for the purchaser, another for the vendor and the third for the referee.

M-3.3 Referee Samples — Referee samples shall consist of a set of individual samples (**M-3.1**) and a composite sample (**M-3.2**) marked for this purpose and shall bear the seals of the purchaser and the vendor. These shall be kept at a place and under conditions agreed to between the purchaser and the vendor.

M-4. NUMBER OF TESTS

M-4.1 Tests for the determination of bacterial count shall be conducted separately on each of the samples constituting a set of individual samples (**M-3.1**).

M-4.2 Tests for the determination of the remaining characteristics shall be conducted on the composite sample (**M-3.2**).

M-5. CRITERIA FOR CONFORMITY

M-5.1 The lot shall be considered as conforming to the requirement of this specification when:

a) each of the test results for bacterial count satisfy the requirements specified in Table 1 (Sl No. viii), and

b) all the test results on the composite sample satisfy the respective requirements as specified in this specification.

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